STUDIES IN EPIDEMIC ENCEPHALITIS (ENCEPHALITIS LETHARGICA)

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In a previous article[1] we presented the results of a number of inoculation experiments in monkeys, and showed that by intracerebral injections of an emulsion of brain tissue in salt solution from a fatal case of epidemic encephalitis we had succeeded in producing in monkeys lesions resembling closely those in man. In that article we described as failures secondary inoculations of brain from monkey to monkey. However, subsequent study of the brain of one of these monkeys (monkey 2) has revealed that we had been led astray by the presence of a gross hemorrhage, which we then interpreted as possibly traumatic. This inoculation we now know to have been successful, both by the presence of typical microscopic lesions at a distance from the hemorrhage, and also by successful inoculation of rabbits as now described.

Successful transmission to monkeys were recorded with the use of Berkefeld N filtrates of both nasopharyngeal washings and nasopharyngeal mucous membrane from encephalitis patients. A secondary inoculation in the monkey was obtained with the filtrate of the nasopharyngeal mucous membrane from a fatal case of epidemic encephalitis. We were unable to produce lesions with the filtrates of the nasopharyngeal mucous membrane from a case of cardiovascular disease.

In this article we present the results of further experiments on monkeys and of a series of experiments on rabbits.

MONKEYS

MONKEY 9 (M. Cynomolgus).—On April 25, 1919, the nasopharynx was painted with filtrate of nasopharyngeal mucous membrane from Wanemacher in 50% glycerin.

May 28: No effect to date.

MONKEY 10 (M. Cynomolgus).—On April 25, 1919, 2 c c of filtrate of nasopharyngeal mucous membrane from fatal case of epidemic encephalitis were injected subdurally.

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[1] New York Med. Jour., 1919, 109, p. 772.

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May 2: Monkey had recovered after a period in which she manifested typical lethargy, general malaise, elevated temperature, and ptosis of the left lid. She was practically moribund at one time. The monkey was allowed to recover for subsequent use in immunity experiments.

MONKEY 4 (M. Rhesus).—On March 22, 1919, 2 c c of filtrate of nasopharyngeal washing from patient, Gordon, with epidemic encephalitis who had recovered were injected subdurally over the left parietal fossa.

March 30: Paresis of both hind legs. The monkey was apathetic; huddled in the corner of the cage and did not respond to stimuli.

April 10: It had fully recovered after a severe illness lasting 10 days.

May 2: Same virulent filtrate of nasopharyngeal mucous membrane as in monkey 10 was injected as follows: 0.5 c c intracerebrally and 1.5 c c subdurally in the left parietal region, 2 c c subdurally in the right parietal region.

September 26: Perfectly normal to date.

MONKEY 17 (Lemur Catta).—On June 25, 1919, it was injected subdurally with 2 c c of filtrate of nasopharyngeal mucous membrane from (control) fatal surgical case.

September 26: No ill effects to date.

RABBITS (CHART 1)

Wherever possible, medium sized rabbits or hares were used to permit of intracranial inoculation by means of simple needle puncture, care being taken to pass just within the skull.

EXPERIMENT 1

RABBITS 3, 4, 7.—On April 25, 1919, they were injected intracranially with filtrate of the nasopharyngeal mucous membrane from a fatal case of epidemic encephalitis.

April 29: Rabbit 4 died. Examination revealed punctate hemorrhages on convexity of brain and intense general congestion; marked meningitis with infiltration of mononuclear leukocytes. Vessels in meninges show perivascular infiltration. There were hemorrhages in cortex, also vessels of cortex.

RABBITS 5, 6.—On April 29 they were injected intracranially with filtrate of brain of rabbit 4.

April 20: Rabbit 5 died. There were hemorrhages over convexity of brain and intense congestion; marked meningitis, mostly mononuclear leukocytes; slight perivascular infiltration; mononuclear infiltration of brain tissue; hemorrhages in cortex.

May 9: Rabbit 6 died. There was moderate congestion; also marked meningitis with mononuclear leukocytes and perivascular infiltration of vessels reaching into cortex from pia.

RABBITS 8, 9, 10.—May 2: The rabbits were injected intracranially with filtrate of brain of rabbit 5.

May 3: Rabbit 8 died. Examination revealed intense congestion; marked meningitis with mononuclear leukocytes mostly; foci of infiltration in cortex with mononuclear and polynuclear leukocytes; areas of intense perivascular infiltration with invasion into surrounding brain tissue.

May 3: Rabbit 9 died. Examination revealed intense congestion; meningitis with mononuclear leukocytes; intense perivascular infiltration of mono-

nuclear leukocytes; present also in basilar ganglions. There were areas of mononuclear infiltration with surrounding zone of necrosis; edema of the brain and marked engorgement of blood vessels.

RABBITS 13 AND 14.—On May 9, 1919, they were injected intracranially with filtrate of brain of rabbit 8.

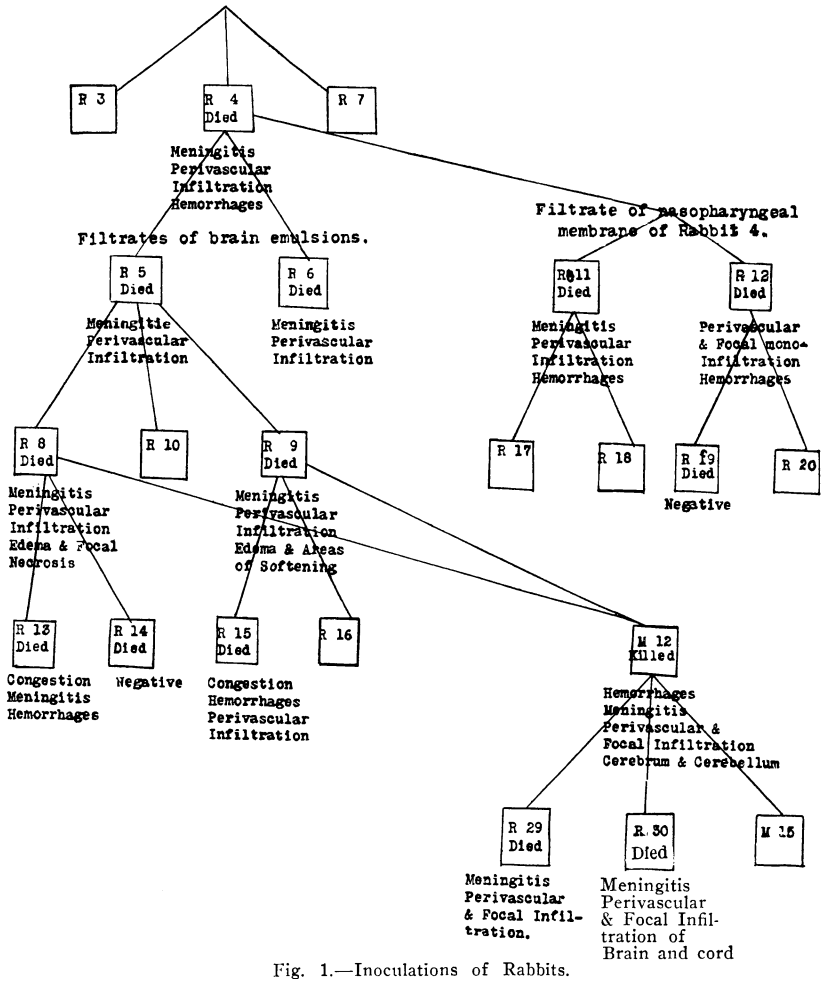

Fig. 1.—Inoculations of Rabbits.

May 15: Rabbit 13 died. Examination revealed intense congestion including congestion of vessels of the pia; one small perivascular hemorrhage; one area of hemorrhage and necrosis.

May 25: Rabbit 14 died. There were intense congestion; small hemorrhages; minute hemorrhages in basilar ganglions; numerous punctate hemorrhages in cerebrum, perivascular infiltration; edema; moderate mononuclear leukocytic meningitis.

RABBITS 15 AND 16.—On May 9 they were injected intracerebrally with filtrate of brain of rabbit 9.

June 6: Rabbit 15 died. The brain showed foci by well marked lesions. Rabbit 16 alive and well (Sept. 26).

MONKEY 12 (Ringtail Lemur Catta).—It was injected subdurally with mixture of filtrates of brains of rabbits 8 and 9.

May 9: Paresis of right arm noted.

May 13: Very ill. Paresis persists. Temperature elevated. Killed. Marked congestion of entire brain. Many punctate hemorrhages around site of injection. No injury to cerebrum. Punctate hemorrhages in midbrain and at base of brain. Lesion in cortex near motor area shows large hemorrhage surrounded by numerous smaller hemorrhages. Tissue is markedly edematous and contains many mononuclear leukocytes. Dilation of perivascular spaces with infiltration of mononuclear leukocytes. Foci of mononuclear leukocytic infiltration in cortex. Region of internal capsule on left side shows multiple hemorrhages with softening. Scattered foci of mononuclear leukocytes. Adjacent brain tissue is edematous. Marked dilation of perivascular lymph spaces with infiltration of mononuclear leukocytes. Meningitis of moderate intensity with mononuclear leukocytes. In cerebellum there is a small microscopic focus of mononuclear leukocytes.

RABBITS 29 AND 30.—On May 21, 1919, they were injected intracranially with filtrate of brain of monkey 12.

May 24: Rabbit 29 died after running course of lethargy for 3 days, during which time it was artificially fed. It was able to swallow if stimulated. Breathing shallow, but regular. Spasticity of both hind limbs noted on first day; flaccid throughout toward end. There were congestion; meningitis with mononuclear cell infiltration; focal infiltration in cortex of mononuclear leukocytes; perivascular and focal infiltration in basilar ganglions; minute microscopic hemorrhages in region of pyramidal fibers in medulla.

June 17: Rabbit 30. Paresis of both hind legs; opisthotonos; apathy.

June 24: Paresis more marked; increasing stupor and death. Very marked typical lesions throughout brain and cord.

EXPERIMENT 2

RABBITS 55 AND 56.—On June 25, they were injected intracranially with filtrate of nasopharyngeal mucous membrane of control fatal surgical case No. 1.

August 2: Rabbit 56 died. Brain negative. Intense bronchopneumonia.

September 26: Rabbit 55 alive and well.

RABBITS 59, 60 AND 61.—On June 15, they were injected intracranially with filtrate of nasopharyngeal mucous membrane of a fatal surgical case No. 2.

September 26: All rabbits alive and well to date.

RABBITS 11 AND 12.—On May 2 they were injected intracranially with filtrate of nasopharyngeal mucous membrane from rabbit 4, which succumbed to intracranial inoculation with filtrate of nasopharyngeal washings of patient.

May 3: Rabbit 11 died. Examination revealed marked congestion; meningitis with infiltration of mononuclear leukocytes; focus of infiltration in cortex with mononuclear leukocytes; area of hemorrhage and edema.

May 6: Rabbit 12 died. Examination revealed punctate hemorrhages over convexity of cerebrum and at base; intense general congestion; small hemor-

rhages in the cortex with a focus of mononuclear leukocytic infiltration; area of mononuclear leukocytes in one of basilar ganglions; and tense parivascular infiltration with mononuclear leukocytes.

RABBITS 17 AND 18.—On May 9, 1919, they were injected intracranially with filtrate of brain of rabbit 11.

May 28: Both alive and well.

RABBITS 19 AND 20.—On May 9, 1919, they were injected intracranially with filtrate of brain of rabbit 12.

May 10: Rabbit 19 died. There was slight congestion of brain; extensive bilateral bronchopneumonia and no abnormalities in brain microscopically.

August 14: Rabbit 20 died. Extensive bronchopneumonia. Mild meningitis.

RABBITS 57 AND 58.—On June 14 they were injected intracranially with filtrate of nasopharyngeal mucous membrane from a normal rabbit.

September 26: Both rabbits alive and well to date.

EXPERIMENT 3

RABBITS 27 AND 28.—On May 5, 1919, they were injected intracranially with filtrate of brain of monkey 2. (This brain had been kept two months in 50 per cent. glycerin solution.)

May 14: Rabbit 27 died. There was slight congestion; no meningitis, no perivascular infiltration, no hemorrhages and no focal infiltration.

May 17: Rabbit 28 died. Examination revealed congestion; moderate meningitis with foci of mononuclear leukocytes; tremendous congestion and dilatation of vessels of pia; very large focus of mononuclear cell infiltration about cortical vessel — vessel practically closed by the infiltration; numerous foci of mononuclear leukocytes scattered throughout cortex and at base of brain; marked congestion of cortical and subcortical vessels.

In every instance the material used for inoculation was cultured aerobically and anaerobically by the usual methods, and also by the Rosenow method, all with negative results. Studies with the Noguchi method of cultivation of filtrable viruses are in progress and the results will be reported later.

CONCLUSIONS

A filtrable virus was obtained from the nasopharyngeal mucous membrane of fatal cases of epidemic encephalitis. The virus is capable of producing in monkeys and rabbits lesions similar to those found in the human brain.

The virus has been carried through four generations in rabbits, transmitted to a monkey in the fifth generation, and then brought back to rabbits.

The virus can be recovered from the nasopharynx of animals inoculated intracranially.

A natural immunity was found in approximately 50% of our rabbits.

An acquired immunity was demonstrated in monkey 4.

A possible connection of this disease with influenza was hinted at in our previous report and studies are now being undertaken to establish such relationship.

NOTE.—Since this publication was submitted, further successful inoculations have been carried out in monkeys and rabbits. Additional control experiments were performed with human and rabbit nasopharyngeal mucous membranes. Typical brain lesions were produced in rabbits by means of filtrates of the nasopharyngeal mucus membrane from several of our positive experimental animals.

One of our strains which in the earlier inoculations manifested a tendency to produce hemorrhages, in subsequent inoculations yielded the more varied lesions found in human brains.

Cerebrospinal fluids from a fatal case of encephalitis caused the disease in rabbits. Transfers from brain to brain through filtrates have been successful in four generations up to the present time. Studies are being continued in this direction.

In the preliminary note appearing in the *Journal of the American Medical Association* for Oct. 4 we describe an organism isolated from the virus.